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14. ABSTRACT
Multiple studies have demonstrated that children with autism spectrum disorders (ASD) have a more oxidized redox status than age-matched controls, and recent studies from project member Dr. S. Jill James (PI, Project 1) have demonstrated that such differences are found even in lymphoblastoid cell lines isolated from children with ASD. Project 2 uses mouse strains and CNS precursor cells with intrinsic differences in oxidative status to identify parameters that can be used to prospectively identify individuals with a more oxidized redox status and also to elucidate the cellular consequences of such a status for cells of the developing central nervous system. The first year's research has focused on identifying core parameters and vulnerabilities using CNS precursor cells that are known to have intrinsic differences in oxidative status despite being isolated from the same animals, thus focusing analysis on outcomes likely to transcend other strain differences. We have defined multiple parameters that are associated with cell-intrinsic differences in redox status (including glutathione status, ATP/ADP ratios, free Ca^{++} levels, and mitochondrial depolarization status and levels of bcl-2, γ-glutamyl cysteinyl synthetase (the rate limiting enzyme in glutathione biosynthesis) and superoxide dismutase 1). Redox status is also predictive of vulnerability to tumor necrosis factor-α and glutamate, two physiological stressors elevated in cerebrospinal fluid of children with ASD.

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2008-2009 Accomplishments - Project 2

Mark Noble, PhD

INTRODUCTION: A wide range of evidence demonstrates that children with autism or autism spectrum disorders (ASD), and also parents of these children, frequently have abnormalities in their redox status that render them more oxidized. Increased levels of oxidized glutathione, as well as other indications of a more oxidized state, have been observed in plasma, serum and urine samples from individuals diagnosed with ASD (Chauhan and Chauhan, 2006; Chauhan et al., 2004; James et al., 2004; James et al., 2006; Ming et al., 2005; Pasca et al., 2006; Sogut et al., 2003; Yorbik et al., 2002; Zoroglu et al., 2004). The prevalence redox abnormalities far exceeds the prevalence of individual mutations that have been associated with ASD (e.g, mutations in the homeobox transcription factor engrailed-2 (Bartlett et al., 2005; Benayed et al., 2005), protein kinase C-beta (Philippi et al., 2005), ataxin-2 binding protein-1 (A2BP1, also referred to as FOX1(Martin et al., 2007)), glutamate receptor 6 (Jamain et al., 2002), methyl-CpG-binding protein 2 (MECP2, which causes Rett syndrome (Shibayama et al., 2004), the mitochondrial aspartate/glutamate carrier (AGC1) (Ramoz et al., 2004), reelin protein (Bartlett et al., 2005; Fatemi et al., 2005; Serajee et al., 2006; Skaar et al., 2005) the promoter region of the c-Met gene (Campbell et al., 2006)). Such a prevalence indicated that increased oxidative status a feature of ASD that is common to multiple genotypes. A more oxidized phenotype would in and of itself be predicted to alter developmental processes and to increase vulnerability to physiological stressors. It is of particular interest in this context that small changes in redox state may have disproportionately large effects on cellular function. Our previous studies have shown that altering glutathione content by as little is 15% is sufficient to greatly increase the vulnerability of neural progenitor cells and neurons to physiological stressors and exposure to suboptimal levels of trophic factors required for cell survival and to alter the response to normal regulators of progenitor cell differentiation (Mayer and Noble, 1994; Smith et al., 2000). For example, a 15% increase in glutathione content is sufficient to cause a 1300% increase in the number of surviving neurons in neuronal cultures exposed to sub-optimal amounts of nerve growth factor. The overall objectives of this research effort are (i) to understand the relationship between altered redox function in children with ASD and the neuropathological changes found in this children, (ii) to provide enhanced means of studying these redox abnormalities and of identifying children who may be more vulnerable to physiological stressors of putative relevance to ASD and (iii) to provide rational approaches to the effective normalization of these metabolic parameters. Project 2 is focused on the hypotheses that redox abnormalities in cells of the hematopoietic system are predictive of redox abnormalities in the CNS, that these abnormalities cause oligodendrocyte progenitor cells to differentiate earlier in development (with subsequent loss of progenitor cells) and that such abnormalities also increase the vulnerability of these progenitor cells to physiological stressors thought to be relevant to ASD pathogenesis.

KEY RESEARCH ACCOMPLISHMENTS

<u>Aim 1</u>: *Analysis of the correlation between redox abnormalities in cells of the peripheral blood and the developing brain.*

a) DoD regulatory review and approval of our UAMS IACUC-approved protocol (months 1-4) **Done**

b) Optimization of methodologies for studying other metabolic aspects of redox balance (months 1-4; while we are awaiting DoD approval we will use established cell lines (that do not require regulatory approval) to optimize all analytic parameters relevant to the remaining components of Task 1)
c) Analysis of the redox status (by dihydrocalcein fluorescence) of peripheral blood cells in multiple mouse strains at two different ages (Months 4-8)
d) Analysis of the redox status of multiple CNS populations in multiple mouse strains (Months 3-12)
e) Analysis of additional metabolic aspects of redox balance in cells of blood and developing brain (Months 3-12)
f) Analysis of proteins that contribute to redox balance in the above cell populations (Months 12-18)

The central question of Aim 1 is to develop better means of identifying cells with different basal redox states. To address this question it is critical to identify a range of redox-related criteria that can be applied to this analysis, so as not to make it dependent upon measurement of single parameters.

• In our work thus far we have first tried to address the problem that train differences may themselves provide differences between cell populations that may or may not be relevant to redox status. Therefore we have focused on goals 1e and 1f in order to first broaden the parameters that will be applied to the populations of 1b and 1c. We have taken advantage of an opportunity afforded by our developmental studies (Power et al., 2002) showing that the glial progenitor cells that give rise to oligodendrocytes isolated from different regions of the CNS of the same animals have different intrinsic redox states. (These cells are referred to in the literature as oligodendrocyte/type-2 astrocyte progenitor cells and also as oligodendrocyte precursor cells, and here abbreviated as O-2A/OPCs) This is an ideal situation in which to define redox-related differences in metabolite and protein expression without concern about differences that might be due to strain differences but unrelated to redox status.

We now have defined a markedly increased range of redox-associated parameters applicable to our further studies. Based on the observations that cortical and corpus callosum O-2A/OPCs exhibit different intracellular redox states, we investigated the physiological basis for these differences. We found multiple differences between cortical and callosal O-2A/OPCs that appear relevant to the differences in redox state between these two cell populations.

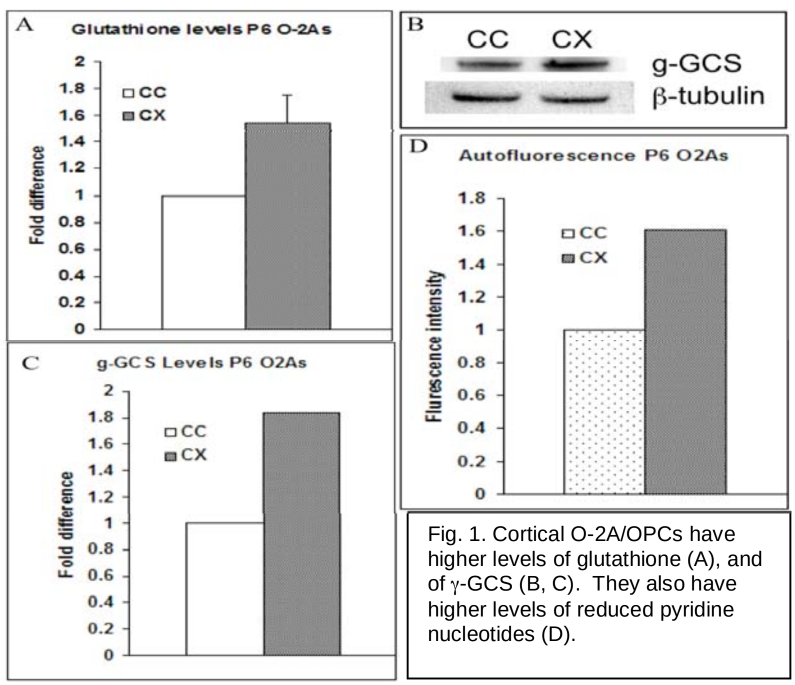
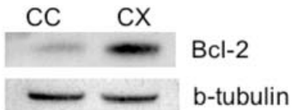


Fig. 1. Cortical O-2A/OPCs have higher levels of glutathione (A), and of γ-GCS (B, C). They also have higher levels of reduced pyridine nucleotides (D).

• Cortical cells have ~2-fold higher levels of reduced thiols, as determined by labeling with monochlorobimane (MCB) (Fig. 1A). As glutathione is the major reduced thiol in cells, MCB is often used as an indicator of glutathione content (Sun et al., 2005). In agreement with this, cortical O-2A/OPCs also have 80% higher levels of γ-glutamyl-cysteinyl-synthase heavy chain (γ-GCS) (Fig. 1B, C), the rate-limiting enzyme in glutathione biosynthesis (Deneke and Fanburg, 1989). Fluorescence analysis also revealed that corpus callosum-derived O-2A/OPCs had lower levels of NADH and NAD(P)H, as compared with cortex-derived cells as determined by

analysis of autofluoresence elicited with an argon laser (488 nm) and multiline ultraviolet light (Figure 1D) (Petit et al., 2001).



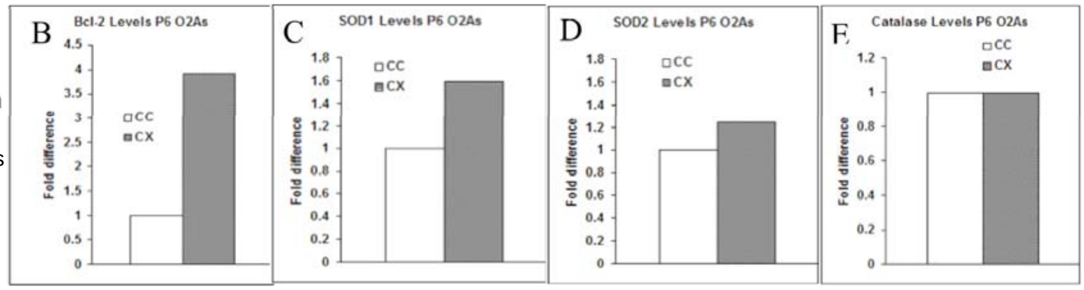


Figure 2 shows that cortical O-2A/OPCs have higher levels of bcl-2 and SOD-1, only slightly higher levels of SOD-2 and similar catalase levels as callosal cells.

• Examination of other proteins known to be associated with cells being more reduced revealed higher levels of bcl-2, the mitochondrial superoxide dismutase (SOD)-1, but not the cytoplasmic SOD-2 or catalase (Figure 2). In particular bcl-2 levels were nearly 4 times higher in cortical cells than in corpus callosum progenitors.

• Our studies also suggest that the mitochondria of cortical O-2A/OPCs may be functionally more active than those in corpus callosum cells. We found that cortical cells have ~20% higher levels of free calcium (as detected by Fura-Red labeling) and also found that cortical progenitors exhibit a 2.5 times higher ADP:ATP ratios than corpus callosum-derived progenitors (Figure 3). Both of these differences are associated with increased mitochondrial activity (Civelek et al., 1996; Gunter et al., 2000), consistent with our analysis with the dye JC-1 that indicates mitochondria in more reduced O-2A/OPCs are more depolarized than those in more oxidized O-2A/OPCs (Smith et al., 2000).

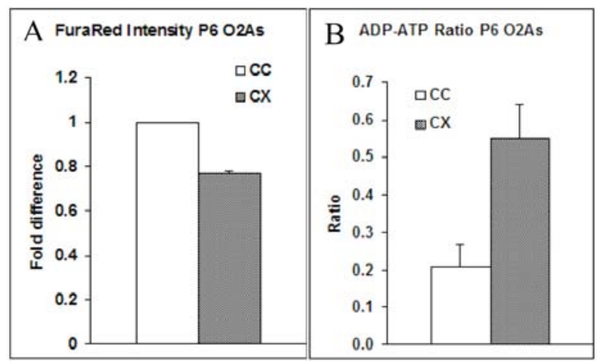


Figure 3 demonstrates that cortical O-2A/OPCs have a higher level of free calcium, as indicated by FuraRed, and also have a higher ratio of ADP:ATP.

<u>Aim 2</u>: Analysis of the biological consequences of strain-dependent redox differences present in oligodendrocyte progenitor cells

a) Analysis of the relationship between redox state of oligodendrocyte progenitor cells isolated from different mouse strains and their ability to undergo division and differentiation in vitro (Months 6-18)
b) Analysis of the correlates of redox status with the time course of myelination in vivo (Months 12-24)
c) Analysis of the consequences of strain-associated differences in redox status for responsiveness of progenitor cells to thyroid hormone as an inducer of oligodendrocyte generation (Months 12-30)
d) Analysis of the consequences of strain-associated differences in redox status for vulnerability of progenitor cells to physiological stressors of putative relevance to ASD pathogenesis (Months 12-30)
e) Analysis of the role of activation of the redox/Fyn/c-Cbl pathway in 2a-d (Months 18-36)
f) Statistical Analysis and manuscript writing: (Years 2 and 3)

As for Aim 1, we have taken advantage of the redox differences in O-2A/OPCs from different regions of the developing CNS to examine vulnerability of cells to physiological stressors, thus defining the issues of Aim 2d in a manner that is more likely to be due to redox differences than to other strain differences. Our results showed that O-2A/OPCs from the cortex (which are more reduced) are less vulnerable to physiological stressors than those from the corpus callosum. We also found, however, that cells of the corpus callosum were heterogeneous in their response to physiological stressors. We therefore focused in greater detail on this population to determine if this heterogeneity in vulnerability to stressors was associated with redox differences and was itself regulated by these redox differences. In order to address

one of the specific physiological stressors that has been shown to be elevated in the cerebrospinal fluid of children with autism, we have focused our attention on tumor necrosis factor-alpha (TNF--α). This protein is produced as part of the inflammatory response, and has long been known to be toxic for oligodendrocyte, the myelin-forming cells of the CNS (which themselves have been suggested to undergo abnormal development in the CNS of children with autism, based on abnormal patterns of myelination seen in these children).

Focusing attention on oligodendrocytes has also enabled us to examine the question of whether a difference in redox status in progenitor cells causes differences in the vulnerability of the differentiated cells they generate. The idea that the metabolic status of a precursor cell may alter the vulnerability of the differentiated cells derived from that precursor cell appears to be a new concept, and supports the hypothesis that redox status may affect cellular function in multiple ways of potential relevant to the pathophysiology of autism spectrum disorders.

• *Heritable vulnerability: The vulnerability of oligodendrocytes to physiological stressors is based on the redox state of their parental O-2A/OPCs*

Virtually nothing is known about the biological underpinnings of differential vulnerability within a population of putatively identical cells. This heterogeneity could represent a truly random dispersion of outcomes in a cellular population, but it is also possible that the seemingly stochastic distribution of outcomes is reflective of identifiable biological differences between surviving and vulnerable cells.

• *Vulnerability to TNF-α is not randomly distributed:*

When we examined oligodendrocyte vulnerability to TNF-α at the clonal level, we found the extent of cell death was non-randomly distributed according to the size of a clone in which an oligodendrocyte was generated Figure 4). Clones in which ≥50% of oligodendrocytes were killed by exposure to 20 ng/ml TNF-α all contained <40 total cells, while in 14/17 clones with >20 cells the majority of oligodendrocytes were not killed by TNF-α.

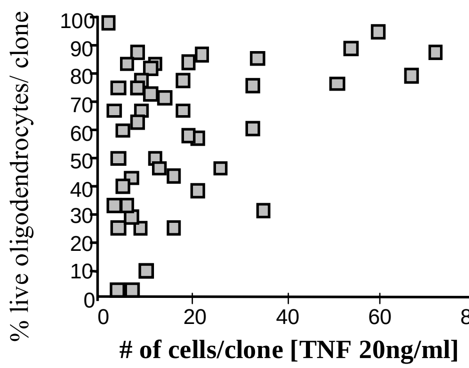


Figure 4 . CC progenitor cells were plated at clonal densities and allowed to differentiate into oligodendrocytes. Clones were treated with TNF at 20ng/ml and the % of live oligodendrocytes in each clone was scored using MTT and DAPI as indicators.

The discovery that TNF-α vulnerability correlates with clonal size raises the possibility that these two characteristics are physiologically related to each other. Our previous studies demonstrated a direct correlation between the intracellular redox state of a progenitor cell and the extent of division occurring in the clone derived from that cell, suggesting a possible explanation for our observations.

• *Vulnerability of oligodendrocytes to TNF-α and glutamate correlates with the intracellular redox state of their ancestral progenitor cell:* We next tested the possibility that the intracellular redox state of a progenitor cell was predictive of the vulnerability of the differentiated cell it generates, applying our previous protocols (Smith et al., 2000) for purifying O-2A/OPCs on the basis of this physiological state. DHCM-Rosamine^{low} progenitors (i.e., those that were more reduced at the time of isolation) gave rise to oligodendrocytes that were resistant to killing by TNF-α, and also were relatively resistant to the background death of oligodendrocytes that normally occurs in these cultures (Figure 5).

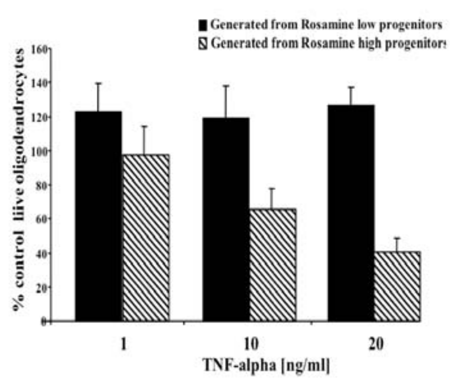


Figure 5: CC derived progenitor cells were separated into rosamine high and rosamine low population using FACS sorting. Oligodendrocytes generated from these two populations were treated with TNF alpha at various doses and the % of live cells was determine using the MTT assay and DAPI staining. Oligodendrocytes derived from the Rosamine low (= more reduced) progenitor cells were significantly less sensitive to TNF- than cells derived from Rosamine high progenitor populations.

Treatment with 1, 10 or 20 ng/ml of

TNF-α had no effect on these cultures, in which viability remained 20% above control values. In striking contrast, DHCM-Rosamine^{high} progenitor cells (i.e, those that were more oxidized at the time of isolation) were vulnerable to TNF-α, with exposure to 10ng/ml TNF-α reducing cell viability by 40% and 20ng/ml reducing viability by 60%. The correlation of progenitor cell redox state with oligodendrocyte vulnerability was not restricted to TNF-α, but was also observed for vulnerability of oligodendrocytes to glutamate-mediated toxicity (data not shown).

• *Pharmacological manipulation of progenitor cell redox state alters vulnerability to TNF-α and glutamate of the oligodendrocytes they generate*: Transient pharmacological manipulation of O-2A/OPC redox state prior to the induction of differentiation indicated the relationship of redox state between progenitor and oligodendrocyte was causally relevant to differences in oligodendrocyte vulnerability to physiological stress. To make O-2A/OPCs more oxidized, we exposed them for 48 hrs to buthionine sulfoximine (BSO, which inhibits glutathione biosynthesis) or to 1mM NAC to make cells more reduced. Cells then were washed and medium containing the compound used to manipulate intracellular redox state was replaced with fresh medium prior to the induction of oligodendrocyte generation. All cultures were exposed to TH for 96 hrs to induce differentiation into oligodendrocytes, after which sensitivity to TNF-α or glutamate was determined. As shown in Figure 6A, O-2A/OPCs exposed to BSO generated oligodendrocytes that were more vulnerable to killing by TNF-α, as compared with untreated cells. Similar results were obtained for glutamate, another physiological stressor that is present at elevated levels when inflammation occurs in the CNS. In contrast, exposure of O-2A/OPCs to 1mM NAC for 48 hrs, followed by induction of oligodendrocyte generation with TH, conferred protection against both glutamate (Figure 6B) and TNF-α (not shown). For example, in control cultures, a significant reduction in cell numbers was seen following exposure to as little as 0.1 mM glutamate, with killing of >60% of cells occurring at glutamate concentrations of 10 mM. In contrast, oligodendrocytes derived from O-2A/OPCs grown transiently in the presence of NAC were not killed at all by 10mM glutamate. Glutamate concentrations of 100mM were required to override the protective effects conferred by transient exposure to NAC prior to the induction of differentiation.

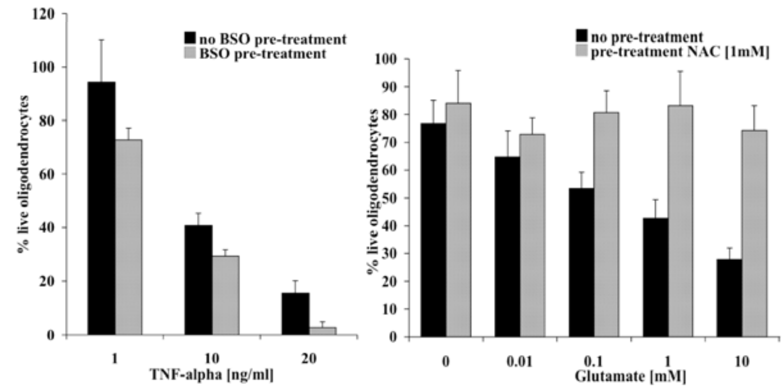


Figure 6. CC derived progenitor cells were pretreated with BSO (A) or NAC (B) for 3 days. Cells were washed and exposed to either TNF-alpha (A) or Glutamate (B). The percent of live oligodendrocytes was scored.

Reportable outcomes: *None*

Conclusions: *Our studies have thus far defined multiple parameters related to differences in cellular redox status and have demonstrated that the redox status of a precursor cell is so important in controlling vulnerability to physiological stressors that it even regulates the vulnerability of the differentiated cell types that it generates. This work now provides an expanded basis for our analysis of strain differences, allowing us to distinguish between changes that correlate with differences in redox status in a manner that is not strain dependent and those changes that may reflect genetic differences between strains. While the latter differences are important to also identify, our present discoveries will make it more efficient to identify differences that are not unique to particular strains and instead have the potential of being of general utility.*

Deliverables: We anticipate 2 publications in major peer-reviewed journals that integrate our present findings with studies on strain differences. In addition, we will analyze the cell lines from autistic children being analyzed in Project 1 in order to obtain information on these additional parameters. The identification of changes in multiple metabolites also provides new targets of potential interest for the methodologies being provided by Project 3. In addition, in the next stage of our analysis we will integrate qnalysis of mechanistic contributions of redox/Fyn/c-Cbl pathway activation to biological outcomes, which may additional parameters of use in defining differences in redox status and also will provide new therapeutic targets for analysis of restoration of normal function and for intervention to protect individuals with vulnerability phenotypes.

Problems encountered and solutions: The major challenge encountered in this portion of our work was in distinguishing strain differences related to redox state from other differences that may represent strain differences that are not generally important in redox state analysis. By focusing analysis on populations that have intrinsic differences in redox status but do not differ genetically we have identified multiple parameters, and also differences in vulnerability to physiological stressors, that cannot be attributed to strain differences that may be irrelevant to achieving our goals. Thus, we have defined a variety of parameters and outcomes related to redox differences in different cellular populations that can now be applied to analysis of strain differences focused on redox parameters.

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NAME Mark D. Noble	POSITION TITLE Professor of Genetics

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR(s)	FIELD OF STUDY
Franklin & Marshall College, Lancaster, P.A.	B.S	1971	Biology & Philosophy
Stanford University, Palo Alto, CA	Ph.D.	1977	Genetics

<u>Previous employment and experience</u>

1977-1981 Honorary Research Assistant, MRC Neuroimmunology Project, Department of Zoology, University College London, London, UK
1981-1983 Research Assistant, Department of Clinical Neurology, Institute of Neurology, London, UK
1984-1987 Senior Research Fellow and Honorary Lecturer, Department of Clinical Neurology, Institute of Neurology, Queen's Square, London, UK
1987-1995 Head, Cellular Neurobiology Laboratory, Ludwig Institute for Cancer Research, Courtauld Building, 91 Riding Street, London, W1P 8BT.
1992-1997 Professor of Cellular and Developmental Biology, Departments of Biochemistry & Molecular Biology and Anatomy & Developmental Biology, University College London, London, UK
1994-1995 Member, Ludwig Institute for Cancer Research, London, UK
1995-2000 Professor of Oncology; Head, Brain Tumor Program; Investigator; Huntsman Cancer Institute and Dept. Oncological Sciences, Univ. of Utah Health Sciences Center, Salt Lake City, Utah.
1998-2000 Co-Director, W.M. Keck Center for Tissue Engineering, University of Utah.
2000-Pres. Professor of Genetics and Professor of Neurobiology and Anatomy, Dept. of Biomedical Genetics, Univ. of Rochester, NY
2005-Pres. Co-Director, Reeve-Richter Center of Research Excellence in Spinal Cord Injury
2007-Pres Director, Univ. Rochester Stem Cell and Regenerative Medicine Institute
2008-Pres Professor of Neurology

<u>Honors</u>

1988: The Jean Monnet Prize of the European Neurological Society (with Damian Wren and Guus Wolswijk)

<u>Federal Government Public Advisory Committee Service</u>

Ad hoc reviewer for multiple NIH study sections; member of Neural Cell Fate (previously MDCN-6) from 2001-2006 (Chair 2005-2006)

<u>Selected peer-reviewed publications (in chronological order out of more than 140)</u>:

1. Raff, M.C., Miller, R.H. & Noble, M. (1983) A glial progenitor cell that develops in vitro into an astrocyte or an oligodendrocyte depending on the culture medium. Nature 303, 390-396.
2. Noble, M. & Murray, K. (1984) Purified astrocytes promote the division of a bipotential glial progenitor cell. EMBO Jl. 3, 2243-2247.
3. Noble, M., Fok-Seang, J. and Cohen, J. (1984) Glia are a unique substrate for the in vitro growth of CNS neurons. J. Neurosci. 4, 1892-1903.
4. Small, R., Riddle, P. and Noble, M. (1987) Evidence for migration of oligodendrocyte-type-2 astrocyte progenitor cells into the developing rat optic nerve. Nature 328, 155-157.
5. Noble, M., Murray, K., Stroobant, P., Waterfield, M. & Riddle, P. (1988) Platelet-derived growth factor promotes division and motility, and inhibits premature differentiation, of the oligodendrocyte-type-2 astrocyte progenitor cell. Nature 333, 560-562.
6. Raff, M.C., Lillien, L.E., Richardson, W.D., Burne, J.F. & Noble, M.D. (1988) Platelet-derived growth factor from astrocytes drives the clock that times oligodendrocyte development in culture. Nature 333, 562-5.
7. Wolswijk, G. & Noble, M. (1989) Identification of an adult-specific glial progenitor cell. Development 105, 387-400.
8. Bögler, O., Wren, D., Barnett, S.C., Land, H. & Noble, M. (1990) Cooperation between two growth factors promotes extended self-renewal, and inhibits differentiation, of O-2A progenitor cells. Proc. Natl. Acad. Sci. U.S.A. 87, 6368-6372.

9. Jat, P.S., Noble, M., Ataliotis, P. Tanaka, Y., Yannoutsos, N., Larsen, L. & Kioussis, D. (1991) Transgenic mice harbouring an H-2K^{b}tsA58 transgene yield conditionally immortalized cell lines. Proc. Natl. Acad. Sci. U.S.A. 88, 5096-5100.
10. Wren, D., Wolswijk, G. & Noble, M. (1992) In vitro analysis of origin and maintenance of O-2A^{adult} progenitor cells J. Cell Biol. 116, 167-176.
11. Wolswijk, G. & Noble, M. (1992) Cooperation between PDGF and FGF converts slowly dividing O-2A^{adult} progenitor cells to rapidly dividing cells with characteristics of their perinatal counterparts. J. Cell Biol. 118, 889-900.
12. Urenjak, J., Williams, S., Gadian, D. and Noble, M. (1993) Proton nuclear magnetic resonance spectroscopy unambiguously identifies different neural cell types. *J. Neurosci.* 13, 981-989.
13. Groves, A.K., Barnett, S.C., Franklin, R.J.M., Crang, A.J., Mayer, M., Blakemore, W.F. & Noble, M. (1993) Repair of demyelinated lesions by transplantation of purified O-2A progenitor cells Nature 362, 453-455.
14. Groves, A.K., Entwistle, A., Jat, P.S. and Noble, M. (1993) The characterisation of astrocyte cell lines that display properties of glial scar tissue. Dev. Biol. 159, 87-104.
15. Bögler, O. & Noble, M. (1994) Measurement of time in oligodendrocyte-type-2 astrocyte (O-2A) progenitors is a cellular process distinct from differentiation or division. Dev. Biol. 162, 525-538
16. Mayer, M. & Noble, M. (1994) N-Acetyl-L-cysteine is a pluripotent protector against cell death and enhancer of trophic factor-mediated cell survival in vitro. Proc. Natl. Acad. Sci. U.S.A. 91, 7496-7500.
17. Pröschel, C., Blouin, M.-J., Gutowski, N.J., Ludwig, R. and Noble, M. (1995) *mLimk1* is predominantly expressed in neural tissues and phosphorylates serine, threonine and tyrosine residues in vitro. *Oncogene* 11, 1271-1281.
18. Ibarrola, N., Mayer-Pröschel, M., Rodriguez-Pena & Noble, M. (1996) Evidence for the existence of at least two timing mechanisms that contribute to oligodendrocyte generation in vitro. Dev. Biol. 180, 1-21.
19. Frangiskakis, J.M., Ewart, A.K., Morris, C.A., Mervis, C.B., Bertrand, J., Robinson, B.F., Klein, B. P., Ensing, G.J., Everett, L.A., Green, E.D., Pröschel, C., Gutowski, N., Noble, M., Atkinson, D.L., Odelberg, S.J. and Keating, M. (1996) *LIM-kinase1* hemizygosity implicated in impaired visuospatial constructive cognition. *Cell* 86, 59-70.
20. Rao, M., Mayer-Pröschel, M and Noble, M. (1998) Isolation of a novel tripotential glial-restricted progenitor cell from embryonic rat spinal cord. Proc. Natl. Acad. Sci. USA . 95, 3996-4001.
21. Yakovlev, A. Y., Boucher, K., Mayer-Pröschel, M. and Noble, M. (1998) Quantitative insight into proliferation and differentiation of O-2A progenitor cells in vitro: The clock model revisited. Proc. Natl. Acad. Sci. U.S.A. 95, 14164-14167.
22. Smith, J., Ladi, E., Mayer-Pröschel, M. and Noble, M. (2000) Redox state is a central modulator of the balance between self-renewal and differentiation in a dividing glial precursor cell. *Proc. Natl. Acad. Sci. U.S.A.* 97, 10032-10037.
23. Mayer-Pröschel, M., Morath, D. and Noble, M. (2001) Are hypothyroidism and iron deficiency precursor cell diseases? *Dev. Neurosci*. 23, 277-286.
24. Gregori, N., Pröschel, C., Noble, M. and Mayer-Pröschel, M. (2002) Tripotential glial-restricted precursor (GRP) cells can be derived from both ventral and dorsal spinal cord, and give rise to bipotential oligodendrocyte-type-2 astrocyte progenitor cells. *J. Neurosci.* 22, 248-256.
25. Power, J., Mayer-Pröschel, M., Smith, J. and Noble, M.(2002) Oligodendrocyte precursor cells from different brain regions express divergent properties consistent with the differing time courses of myelination in these regions. *Dev. Biol.* 245, 362-375.
26. Dietrich, J., Noble, M. and Mayer-Pröschel, M. (2002) Characterization of A2B5+ glial precursor cells from cryopreserved human fetal brain progenitor cells. *Glia*. 40, 65-77.
27. Noble, M. Arhin, A., Gass, D. and Mayer-Pröschel, M. (2003) The cortical ancestry of oligodendrocytes: Common principles and novel features. *Devel. Neurosci* 25, 217-233.
28. Hill, C.E., Pröschel, C., Noble, M., Mayer-Pröschel, M, Gensel, J.C., Beattie, M.S. and Bresnahan, J.C. (2004) Acute transplantation of glial restricted precursor cells into spinal cord contusion injuries: survival, differentiation and effects on lesion environment and axonal regeneration. *Exp. Neurol.* 190, 289-310.
29. Dietrich, J., Lacagnina, M., Gass, D., Richfield, E., Mayer-Pröschel, M., Noble, M., Torres, C. and Pröschel, C. (2005) *EIF2B5* mutations compromise generation of GFAP^{+} astrocytes from neural precursors in Vanishing White Matter leukodystrophy *Nature Medicine* 11, 277-283.
30. Jordan, C.T., Guzman, M.L. and Noble, M. (2006) The next challenge for targeted therapy in malignancy: Selective eradication of cancer stem cells. *New Engl. J. Med.* **355**, 1253-1261

31. Davies, J. E., Huang C., Proschel, C., Noble, M., Mayer-Proschel, M. and Davies, S. J. (2006) Astrocytes derived from glial-restricted precursors promote spinal cord repair. *J Biol.* 5:e7
32. Hyrien O., Ambeskovic I., Mayer-Proschel M., Noble M., Andrei Yakovlev. Stochastic modeling of oligodendrocyte generation in cell culture: model validation with time-lapse data (2006) *Theoretical Biology and Medical Modelling*.17:3-21
33. Li, Z., Dong, T., Pröschel, C. and Noble, M. (2007) Chemically diverse toxicants converge on Fyn and c-Cbl to disrupt precursor cell function. PLoS Biology 5:e35.
34. Davies JE, Proschel C, Zhang N, Noble M, Mayer-Proschel M and Davies SA Transplanted astrocytes derived from BMP or CNTF treated glial restricted precursors have opposite effects on recovery and allodynia after spinal cord injury. (2008) *J Biol.* 7*:e24.* PMC Journal – In Process
35. Han R.; Yang Yin M.; Dietrich J.; Luebke A.; Mayer-Proschel M. & Mark Noble (2008) Systemic 5-fluorouracil treatment causes a syndrome of delayed myelin destruction in the CNS. *J Biol* 7:e12. PMCID 2397490
36. Ratan, R.R. and Noble, M. (2008) Novel multi-modal strategies to promote brain and spinal cord injury recovery. *Stroke* (DOI: 10.1161/STROKEAHA.108.534933). PMC2655641

C. Research Support

CO19772 3771N Ratan (PI) New York State Spinal Cord Injury Research Program 05/01/2004 - 04/30/2009
(Sub Contract from Winifred Burke Medical Research Institute, Inc.) *no cost extension until 4/30/10*
New York State Spinal Cord Injury Research Program – Admin Core and Cell Therapies
Development of improved therapies for spinal cord injury Role: PI for Univ. Rochester component of the consortium.

RO1 ES012708 Noble (PI) NIH 01/01/2006 – 12/31/2010
Low-level toxicant perturbation of neural cell function
This project is based on the discovery that chemically diverse toxicants converge on disruption of a common molecular pathway, leading to amplified degradation of specific receptor tyrosine kinases and suppression of signaling along these pathways. Through activation of this receptor degradation pathway neural precursor cell division is inhibited. The project is focused on a detailed mechanistic analysis of this inhibition.

RO1 NS39511 Continuation Hyrien (PI) NIH (Role = CoI) 06/01/2007 – 03/31/2012
Stochastic modeling of multi-type cell systems
Development of novel quantitative approaches to analysis of precursor cell differentiation at the clonal level.

W81XWH-07-1-0601 Fisher (PI) DOD (Role = CoI) 09/01/2007-08/31/2012
Early Diagnosis, Treatment and Care of Cancer Patients
Development of novel chemosensitivity agents for leukemia.

BCTR0707697 Noble (PI) The Susan G. Komen Breast Cancer Foundation 10/01/2007 –10/22/2009
Adverse neurological consequences of breast cancer treatments: Causes and prevention *no cost ext. pending*
Goals of this research are to investigate tamoxifen and 5-FU toxicity in the CNS and identify prognostic indicators of vulnerability to these chemotherapeutic agents.

R21 HD055550-01-A1 Mayer-Pröschel (PI) NIH (Role = unpaid collaborator) 04/01/2008 – 03/31/2010
Glial dysfunction in Ataxia Telangiectasia
This proposal is focused on the characterization of a novel cellular target we have identified that is affected in the genetic disease Ataxia telangiectasia (AT).

1R21 MH083581-01 Gelbard (PI) NIH (Role = unpaid collaborator) 04/01/2008 – 03/31/2010
The Axon-Oligodendrocyte Precursor Synapse in NeuroAIDS
The major goal of this project is to investigate glutamatergic signaling between unmyelinated axons and oligodendrocyte precursor cells in response to the HIV-1 regulatory protein Tat.

Award letter dated March 28th, 2008 Noble (PI) 06/01/2008 – 05/31/2009
Hunter's Hope Foundation *no cost extension until 5/31/10*
Stem cell therapy for Krabbe's disease: The problem of chemotherapy-mediated damage to the CNS.

R01CA131385 Noble (PI) NIH 07/01/2008 – 06/30/2013
CNS vulnerability to systemic chemotherapy: Causes and prevention
This research investigates biological and mechanistic foundations for adverse effects of systemic chemotherapy, both to discover means of protecting against such events and to develop means of identifying individuals at increased risk for adverse events.

R21 AG030711-01A1 Noble (PI) NIH 07/01/2008 – 06/30/2010
White Matter Damage in Alzheimer's disease: New cellular targets and mechanisms
Analysis of the mechanisms by which amyloid ß protein causes damage to oligodendrocyte lineage cells in

Alzheimer's disease. This research will help in identifying new means of protecting against amyloid ß toxicity.
R01 CA134839-01 Hyrien (PI) NIH (Role = coI) 09/01/2008 - 08/31/2013
Statistical Inference on Chemotherapy Effects from Flow Cytometry Data
Development of quantitative approaches to the analysis of chemotherapy-mediated damage to normal tissue.
AS073218 Noble (PI) DOD 09/15/2008 – 09/14/2011
Redox Abormalities as a Vulnerability Phenotype for Autism and Related Alterations in CNS Development.
Award Letter Dated September 17th, 2008 Noble (PI) Autism Speaks 10/01/2008 – 09/30/2011
Vulnerability phenotypes and susceptibility to environmental toxicants: from organism to mechanism
Aim 1 tests the related hypotheses that genetically-based differences in oxidative status are associated with differences in vulnerability to physiological stressors at the cellular and organism level, with even greater increases in vulnerability to combinations of physiological stressors. Aim 2 tests the hypothesis that the redox/Fyn/c-Cbl pathway is a point of necessary mechanistic convergence for increases in vulnerability caused by a more oxidized metabolic status.
N08P-014 Sell (PI) NYS DOH (Sub Contract from Wadsworth/Ordway Res. Inst.) 11/01/2008 – 10/31/2009
Stem Cells and Aging Consortium. Role: PI for University of Rochester subcontract to Wadsworth/Ordway Research Inst.
N08P-024 Noble (PI) NYS DOH 11/01/2008 – 10/31/2009
Clinical Translation in Stem Cell Medicine: From Principles to Practice
Planning grant for the development of a translational stem cell medicine consortium. therapies.
C023691 (Mayer-Pröschel) NYS DOH/Spinal Cord Injury Research Board (Role: Co-I)10/01/08–09/30/2012
Specific astrocyte subtypes for SCI repair without allodynia
Aim 1 tests the hypotheses that GDA^{BMP} transplantation, but not GRP cell transplantation, (i) protects against cell death in the injured spinal cord, maintains/promotes division of precursor cells required for tissue maintenance and repair and suppresses inflammation. Aim 2 tests the hypothesis that astrocytes generated by exposure of embryonic spinal cord GRP cells to BMP are more effective in repairing the damaged spinal cord than astrocytes generated by exposure to BMP of stem or progenitor cells from other CNS regions or from older animals. Aim 3 tests the hypothesis that pre-differentiation of stem or progenitor cells prior to transplantation is required in order to prevent neuropathic pain syndromes.
N08G-048 (Bohmann) NYS DOH (Role: Co-I) 01/01/2009 – 12/31/2011
Nrf2 as a regulator of stem and progenitor cell function
This research will functionally test the hypothesis of Nrf2's critical role in stem and progenitor cell regulation. The experimental program will seek further support for the redox mechanism of precursor cell regulation and establish this process in *Drosophila*.

Completed Research Support (during the last 3 years)

CO202942 Noble (PI) New York State Spinal Cord Injury Research Program 01/01/2006 – 12/31/2007
Remyelination of SCI: Overcoming the inhibitors (no cost extension till 12/31/08)
The major goals of this project arise from observations that glial scar tissue inhibits the migration of cells required for repair of demyelinating damage in the CNS. This project seeks to identify the molecular basis for this inhibition, thus potentially enabling enhancement of remyelination. Role: PI
Award letter dated 12/11/06 Noble (PI) Cure Autism Now Foundation 02/05/2007 – 02/04/2009
Cellular, physiological and molecular mechanisms underlying alterations
CNS development caused by exposure to clinically relevant levels of mercury containing compounds
This research effort tests the hypotheses that there are multiple adverse effects on cells of the developing CNS of clinically relevant levels of organomercurials, and that these are modulated by the organism's redox state.
CO23056 Guzick (PI) New York State Department of Health 04/01/2008 – 03/31/2009
NY Stem Grant for Institutional Development of Stem Cell Research Capabilities
Subproject 9S: Low-level toxicant perturbation of neural cell function
Supplemental funding for analysis of the role of the redox/Fyn/c-Cbl pathway in precursor cell function.
RO1 NS44701 Noble (PI) NIH 05/01/2002-04/30/2006
Oligodendrocytes & precursors: toxicity of chemotherapy (no cost extension till 6/30/07)
To evaluate and repair neurological damage that occurs during an after course of chemotherapy. Role: PI
RO1 HD39702 Noble (PI) NIH 07/01/2001-06/30/2006
CNS precursor cell dysfunction in developmental maladies (no cost extension till 6/30/07)
This proposal tests the hypothesis that the basis for childhood disorders of neurological development is disruption of specific steps in the development of the precursor cells that give rise to the differentiated cell types of the central nervous system (CNS). Role: PI